

# The Functional Form of Angular Forces around Transition Metal Ions in Biomolecules

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## Abstract

A method for generating angular forces around  $\sigma$ -bonded transition metal ions is generalized to treat  $\pi$ -bonded configurations. The theoretical approach is based on an analysis of a ligand-field Hamiltonian based on the moments of the electron state distribution. The functional forms that are obtained involve a modification of the usual expression of the binding energy as a sum of ligand-ligand interactions, which however requires very little increased in CPU time. The angular interactions have simple forms involving sin and cos functions, whose relative weights depend on whether the ligands are  $\sigma$ - or  $\pi$ -bonded. They describe the ligand-field stabilization energy to an accuracy of about 10%. The resulting force field is used to model the structure of small clusters, including fragments of the copper blue protein structure. Large deviations from the typical square copper coordination are found when  $\pi$ -bonded ligands are present.

Running title: Angular Forces around Ions

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## I. INTRODUCTION

Atomic-level simulations of proteins interacting with transition-metal ions have the promise of elucidating a wide variety of biophysical phenomena (Kaim, 1994). Such simulations can help establish both the native structure and reaction paths of metalloproteins, and explain the roles of metal ions in protein folding and stability. Similar applications can be seen for simulations of metal ions interacting with DNA and RNA. However, the utility of such simulations depends critically on the availability of accurate but computationally tractable force fields for metals interacting with proteins. There are no quantitatively accurate force fields for these purposes, and it is probably not possible to construct force fields of a simple enough form to be useful that have quantitative accuracy. However, one can hope to generate force fields that include the basic physical effects at a level of accuracy sufficient to ascertain chemical trends as metals or ligands vary. The situation in this regard is best for “simple” metal ions that have no partly occupied shells. Here an ionic picture based on electrostatics, supplemented by empirical repulsive forces, may hope to treat the most important physical effects. Even here though, care should be taken, since the bond is not completely ionic but has some partial covalent character. For transition metals, the situation is much more difficult. The ligand-field splitting of the  $d$ -shell leads to important electronic contributions which cannot be ignored. These are manifested, for example, in the typical square or tetragonal coordination of  $\text{Cu}^{2+}$  complexes. Such effects cannot be described by radial interactions, but rather require the introduction of angular forces describing the energetics of the transition-metal  $d$ -shell. Most existing methods for including angular forces in simulations of metal ions have used assumed functional forms (Comba et al., 1995, Allured et al., 1991, Timofeeva et al., 1995, Wiesemann et al., 1994, Sayle et al., 1995, Sayle et al., 1997) based, for example on the observed structures of small complexes. A recently developed method (Landis et al., 1998) treats the environment-dependent energetics of transition metals via a valence-bond approach. This method appears promising for covalently bonded metals. However, the bonding state of transition metals in proteins is may be rather different from the  $sd^n$  hybridized configuration assumed in that work. In the present analysis, we seek to develop a method suitable for more ionic configurations.

We have recently shown (Carlsson, 1998) how the ligand-field splitting effects of  $\sigma$ -bonded transition metal ions can be described by a force field of a fairly simple form. One starts with an explicitly quantum-mechanical form for the electronic energy, and then then solves the quantum-mechanical problem with systematic approximation methods to extract the real-space description of the electronic bonding energy. A second-order treatment of the hybridization terms between the ligand and metal orbitals is used to generate a ligand-field Hamiltonian for the  $d$ -electrons. The electronic bonding energy of this Hamiltonian is analyzed in terms its moments. The moment analysis of the energy gives a “semiclassical” energy function with a simple trigonometric angular form. It is not precisely an additive function of ligand-ligand interactions, but is nearly as simple computationally. This energy function gives quite accurate energy results for  $\sigma$ -bonded ligands, with less than 10% error in the ligand-field stabilization energy (defined below).

This approach is extended in the present paper, in two ways. First, we develop energy functions which treat  $\pi$ -bonded ligands as well. This results in new and distinct angular forms. Second, we present results for the lowest-energy structures of small clusters, which

are a guide to understanding the bonding preferences of transition metals in proteins.

The organization of the remainder of the paper is as follows. Section II develops the formalism underlying the angular forces. Section III gives tests of the functional form of the angular forces by comparing with results from diagonalization of simple cluster Hamiltonians. Section IV gives the small-cluster results for ideal examples, and for an analogue of the copper environment in blue-copper proteins.

## II. MODEL

We model the electronic structure of the environment of a single transition metal ion in terms of orbitals  $|L, \mu\rangle$  localized on the ligands and  $|M, \nu\rangle$  localized on the single metal ion. The orbitals taken to be orthogonal for simplicity of calculation. Here “L” denotes a particular ligand atom and the  $|L, \mu\rangle$  are distinct orbitals on that atom. The Hamiltonian takes the following form:

$$\begin{aligned} \hat{H} = & \sum_{L,\mu} \varepsilon_{L,\mu} |L, \mu\rangle \langle L, \mu| + \sum_{\nu} \varepsilon_{M,\nu} |M, \nu\rangle \langle M, \nu| \\ & + \sum_{L,\mu,\nu} \left[ h_{LM}^{\mu\nu} |L, \mu\rangle \langle M, \nu| + h_{ML}^{\nu\mu} |M, \nu\rangle \langle L, \mu| \right] . \end{aligned} \quad (1)$$

Here the  $\varepsilon$  terms are on-site energies for the orbitals, and the  $h$  terms are hybridization energies between the ligand orbitals and the metal orbitals. We ignore electron-interaction terms as well as explicit electrostatic effects. The energy associated with this Hamiltonian can then be obtained by diagonalizing its matrix as given in the  $|L, \mu\rangle - |M, \nu\rangle$  basis, and taking the sum of the energies of the occupied eigenvectors.

To obtain a real-space description of the energetics of this Hamiltonian, we make the simplifying approximation that the dimensionless ratios  $|h_{LM}^{\mu\nu}|/(\varepsilon_{M,\nu} - \varepsilon_{L,\mu})$  are small and can be used as expansion parameters. This will be the case if the bonding is primarily ionic as opposed to covalent. In the case of transition metal ions, this allows a great simplification by the application of ligand-field theory, which gives an effective Hamiltonian for the  $d$ -shell:

$$\hat{H}_d = \sum_{\mu,\nu} h_d^{\mu\nu} |M, \mu\rangle \langle M, \nu| , \quad (2)$$

where

$$h_d^{\mu\nu} = \sum_{L,\eta} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} h_{ML}^{\mu\eta} h_{LM}^{\eta\nu} . \quad (3)$$

Note that we have assumed all of the  $d$ -orbitals on the transition metal ion to have the same energies before ligand-field effects are “turned on”.

In a fully quantum-mechanical ligand-field-theory calculation, one would numerically diagonalize the matrix  $\hat{H}_d$ . In order to obtain a force field with a nearly classical form, we instead work with the moments of this matrix, defined in terms the traces of its powers, as follows. The first moment is defined as the average energy of the  $d$ -complex, or

$$\begin{aligned} \bar{\varepsilon} = & (1/5) \text{Tr} \hat{H}_d = (1/5) \sum_{L,\eta} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} \sum_{\mu} h_{ML}^{\mu\eta} h_{LM}^{\eta\mu} \\ = & (1/5) \sum_{L,\eta} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} g_{\eta\eta} \end{aligned} \quad (4)$$

where

$$g_{\eta,\eta'} = \sum_{\mu} h_{L'M}^{\eta'\mu} h_{ML}^{\mu\eta} \quad . \quad (5)$$

Thus  $\bar{\varepsilon}$  is given as a sum of independent contributions from the ligands. Because the  $d$ -shell by itself has spherical symmetry, the contribution from each ligand is a radial function (no angular dependence) of the metal-ligand distance. Our major interest is in the angular terms resulting from the ligand-field splitting, so we do not consider the  $\bar{\varepsilon}$  term further.

The width  $W$  of the  $d$ -complex corresponding to the ligand-field splitting can in the simplest picture be described in terms of the second moment or variance  $\delta\varepsilon^2$  of the eigenvalues of  $\hat{H}_d$ . We expect that  $W \propto \sqrt{\delta\varepsilon^2}$ . We can obtain a simple real-space form for  $\delta\varepsilon^2$  as follows: First we note that  $\delta\varepsilon^2 = (1/5) \sum_n (\varepsilon_n - \bar{\varepsilon})^2 = (1/5) \text{Tr}(\hat{H}_d - \bar{\varepsilon}\hat{I})^2$  where the  $\varepsilon_n$  are the eigenvalues of  $\hat{H}_d$ . From Eqs. (2), (3), and (5), we see that

$$\begin{aligned} \text{Tr}\hat{H}_d^2 &= \sum_{\mu,\nu} h_d^{\mu\nu} h_d^{\nu\mu} \\ &= \sum_{L,\eta,L',\eta'} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} (\varepsilon_d - \varepsilon_{L',\eta'})^{-1} \sum_{\mu} h_{L'M}^{\eta'\mu} h_{ML}^{\mu\eta} \sum_{\nu} h_{LM}^{\eta\nu} h_{ML'}^{\nu\eta'} \\ &= \sum_{L,\eta,L',\eta'} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} (\varepsilon_d - \varepsilon_{L',\eta'})^{-1} g_{\eta\eta'}^2 \quad . \end{aligned} \quad (6)$$

Then the variance is

$$\begin{aligned} \delta\varepsilon^2 &= (1/5) \text{Tr}(\hat{H}_d - \bar{\varepsilon}\hat{I})^2 = (1/5) (\text{Tr}\hat{H}_d^2 - 5\bar{\varepsilon}^2) \\ &= (1/5) \sum_{L,\eta,L',\eta'} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} (\varepsilon_d - \varepsilon_{L',\eta'})^{-1} [g_{\eta\eta'}^2 - (1/5)g_{\eta\eta}g_{\eta',\eta'}] \quad . \end{aligned} \quad (7)$$

We will focus on the ligand-field stabilization energy, which is defined as the sum of the energies of the occupied orbitals relative to  $\bar{\varepsilon}$ , or  $E_{\text{LFSE}} = \sum'_n (\varepsilon_n - \bar{\varepsilon})$ , where the sum is over only the occupied eigenfunctions. We assume that  $E_{\text{LFSE}}$  is proportional to the ligand-field splitting  $W$ , and also identify  $W$  with the standard deviation  $\sqrt{\delta\varepsilon^2}$ . Thus our expression for the ligand-field stabilization energy has the form

$$E_{\text{LFSE}} = -\left\{ \alpha/5 \sum_{L,\eta,L',\eta'} (\varepsilon_d - \varepsilon_{L,\eta})^{-1} (\varepsilon_d - \varepsilon_{L',\eta'})^{-1} [g_{\eta\eta'}^2 - (1/5)g_{\eta\eta}g_{\eta',\eta'}] \right\}^{1/2} \quad , \quad (8)$$

where  $\alpha$  is a dimensionless constant which will later be used as a fitting parameter.

The accuracy of this form will be demonstrated in the next section via specific numerical experiments. At present, we will show how this form for  $E_{\text{LFSE}}$  results in an expression for the energy in terms of simple angular interactions between the ligands. We thus need to develop analytic forms for the  $g_{\eta\eta'}$ . First we note that we can write

$$\begin{aligned} g_{\eta\eta'} &= \langle L, \eta | \hat{H} \hat{P}_d^2 \hat{H} | L', \eta' \rangle \\ &= \langle \Psi | \Psi' \rangle \quad , \end{aligned} \quad (9)$$

where  $\hat{P}_d = \sum_{\mu} |M, \mu\rangle \langle M, \mu|$  is the projection onto the  $d$ -subspace of the transition metal ion. Here we define  $|\Psi\rangle = \hat{P}_d \hat{H} |L, \eta\rangle$  and we have used the relation  $\hat{P}_d^2 = \hat{P}_d$ , which follows from  $\hat{P}_d$ 's being a projection operator.

Consider first the case where both the  $|L, \eta\rangle$  and  $|L', \eta'\rangle$  orbitals have  $\sigma$ -character with respect to the metal ion. Then they couple only to the  $d$ -orbitals  $|M, \sigma\rangle$  and  $|M, \sigma'\rangle$  that have  $\sigma$ -character with respect to the bond axes, so that  $|\Psi\rangle = h_\sigma|M, \sigma\rangle$  and  $|\Psi'\rangle = h'_\sigma|M, \sigma'\rangle$ , where  $h_\sigma$  and  $h'_\sigma$  are the appropriate coupling strengths. Thus  $g_{\eta\eta'} = h_\sigma h'_\sigma \langle M, \sigma | M, \sigma' \rangle$ , and the angular dependence is contained in the last inner product. But this is simply a matrix element of a rotation about  $M$  which carries  $L$  into  $L'$ . We note that  $|M, \sigma\rangle$  is equivalent to  $|M, m = 0\rangle$ , where  $m = 0$  denotes the angular dependence of the spherical harmonic  $Y_{2m}$ . Choosing our coordinate system so that  $L$  is along the  $z$ -axis and  $L'$  is in the  $z$ - $x$  plane, we find that

$$\begin{aligned} \langle M, \sigma | M, \sigma' \rangle &= D_{00}^{(2)}(0, \theta, 0) \\ &= (3 \cos^2 \theta - 1)/2 \end{aligned} \quad (10)$$

where the  $D$ -term is a matrix element of the  $l = 2$  representation of the rotation group, and the second equality follows from the explicit formulas of the  $D$ -terms given in Ref. (Wigner, 1959). In summary, for  $\sigma$ -bonded ligands,

$$g_{\eta\eta'} = h_\sigma h'_\sigma (3 \cos^2 \theta - 1)/2 \quad . \quad (11)$$

For the case of a  $\pi$ -bonded orbital  $L$  and a  $\sigma$ -bonded orbital  $L'$ , we consider only the case in which the axis of the orbital lies along the circle connecting  $L$  and  $L'$ ; if it is perpendicular to this circle,  $L$  and  $L'$  have different inversion symmetries so their coupling vanishes. We write  $|\Psi\rangle = h_\pi|M, \pi\rangle$  where  $|M, \pi\rangle = (1/\sqrt{2})(-|M, m = 1\rangle + |M, m = -1\rangle)$  and  $m$  the usual azimuthal angular momentum index for the spherical harmonics. (The “ $-$ ” sign comes from the definition of the spherical harmonics). Then, again choosing a coordinate system in which  $L$  is along the  $z$ -axis and  $L'$  is in the  $z$ - $x$  plane, and following reasoning parallel to the  $\sigma$ -bonded case, we see that

$$\begin{aligned} \langle M, \pi | M, \sigma' \rangle &= (1/\sqrt{2})[-D_{10}^{(2)}(0, \theta, 0) + D_{-10}^{(2)}(0, \theta, 0)] \\ &= \sqrt{3} \sin \theta \cos \theta \quad . \end{aligned} \quad (12)$$

and

$$g_{\eta\eta'} = \sqrt{3} h_\pi h'_\sigma \sin \theta \cos \theta \quad . \quad (13)$$

Finally we turn to the case of two  $\pi$ -bonded orbitals. To obtain the subsequent results, it is sufficient to consider the case 1) in which the orbitals are parallel to the arc connecting  $L$  and  $L'$ , and the case 2) in which they are perpendicular to it. By reasoning similar to that above, one sees that in case 1)

$$\begin{aligned} \langle M, \pi | M, \pi' \rangle &= (1/2)[D_{11}^{(2)}(0, \theta, 0) + D_{-1-1}^{(2)}(0, \theta, 0) - D_{1-1}^{(2)}(0, \theta, 0) - D_{-11}^{(2)}(0, \theta, 0)] \\ &= \cos 2\theta \end{aligned} \quad (14)$$

and

$$g_{\eta\eta'} = h_\pi h'_\pi \cos 2\theta \quad . \quad (15)$$

In case 2),

$$\begin{aligned}\langle M, \pi | M, \pi' \rangle &= (1/2) [D_{11}^{(2)}(0, \theta, 0) + D_{-1-1}^{(2)}(0, \theta, 0) + D_{1-1}^{(2)}(0, \theta, 0)l + D_{-11}^{(2)}(0, \theta, 0)] \\ &= \cos \theta\end{aligned}\quad (16)$$

and

$$g_{\eta\eta'} = h_\pi h'_\pi \cos \theta \quad . \quad (17)$$

Thus, combining Eqs. (8), (11), (13), (15), and (17), we find the following explicit form for  $E_{\text{LFSE}}$  as a sum of ligand-ligand interactions:

$$E_{\text{LFSE}} = - \sqrt{\sum_{L, \eta, L', \eta'}} U(L, \eta; L', \eta') \quad (18)$$

where the ligand-ligand interaction  $U$  is given as follows:

For  $\sigma$ - $\sigma$  interactions,

$$U(L, \eta; L', \eta') = (\alpha/5) e_\sigma(r) e_\sigma(r') u_{\sigma\sigma}(\theta) \quad (19)$$

for  $\pi$ - $\sigma$  interactions,

$$U(L, \eta; L', \eta') = (\alpha/5) e_\pi(r) e_\sigma(r') u_{\pi\sigma}(\theta) \quad (20)$$

and for  $\pi$ - $\pi$  interactions,

$$U(L, \eta; L', \eta') = (\alpha/5) e_\pi(r) e_\pi(r') u_{\pi\pi}(\theta) \quad (21)$$

where

$$e_\sigma(r) = h_\sigma^2 / (\varepsilon_d - \varepsilon_{L, \eta}), \quad (22)$$

$$e_\pi(r) = h_\pi^2 / (\varepsilon_d - \varepsilon_{L, \eta'}), \quad (23)$$

$$u_{\sigma\sigma}(\theta) = (9 \cos^4 \theta - 6 \cos^2 \theta + 1/5)/4, \quad (24)$$

$$u_{\pi\sigma}(\theta) = (-3 \cos^4 \theta + 3 \cos^2 \theta - 2/5), \quad (25)$$

$$u_{\pi\pi}(\theta) = (4 \cos^4 \theta - 3 \cos^2 \theta + 1/5) \quad . \quad (26)$$

In each case,  $U$  is given as a product of radial terms involving the two ligands, and a simple angular function. (Note that although denoted an interaction here,  $U$  does not have units of energy because of the square root in Eq. (18)). In the calculation of the terms involving  $\pi$ - $\sigma$  interactions, each term involves a sum over two  $\pi$ -orbitals, parallel and perpendicular to the arc connecting the two ligands. In the latter case,  $g_{\eta\eta'}$  in Eq. (8) vanishes, but  $g_{\eta\eta}$  and  $g_{\eta'\eta'}$  do not. In the calculation of the terms involving  $\pi$ - $\pi$  interactions, one has a similar scenario except that one sums over two pairs of  $\pi$ -orbitals.

These forms are plotted out in Figure 1a. Note that the  $\sigma$ - $\sigma$  interactions are fairly similar in form to the  $\pi$ - $\pi$  interactions, both having pronounced minima at  $180^\circ$  (as well as the physically irrelevant one at  $0^\circ$ ), and a shallower minimum at  $90^\circ$ . The  $\sigma$ - $\pi$  interaction is

complementary to these, having minima at  $45^\circ$  and  $135^\circ$ . We shall see later that these differences lead to large differences in ground-state structures of small clusters. For comparison, we show in Figure 1b an empirical angular interaction curve (Comba et al., 1995) assumed in some previous calculations of small transition-metal structures. The angular dependence is based on the observed square structure of small complexes of the transition metals of interest, and is proportional to  $\sin^2 2\theta$ . Since the metals which have square coordination generally have predominantly  $\sigma$ -bonds to their neighbors, the most relevant comparison is to the  $\sigma$ - $\sigma$  curve in Figure 1a. We see that the behavior is quite different. The empirical curve has equivalent minima at  $180^\circ$  and  $90^\circ$ , while in the theoretical curve the  $180^\circ$  minimum is much deeper.

### III. TESTS OF FUNCTIONAL FORM

In order to evaluate the accuracy of the semiclassical form of Eq. (8) for the ligand-field stabilization energy, we have performed explicit tests for small clusters. These clusters consist of a central transition-metal ion with four neighbors placed at random orientations at random distances relative to the central ion. We consider only the minority-spin orbitals, as these determine  $E_{\text{LFSE}}$  for high-spin late transition metals. Four electrons are placed in these states, corresponding to  $\text{Cu}^{2+}$ ; other band filling values gives similar results. The random distances are taken into account by varying the couplings  $h_\sigma$  and  $h_\pi$  uniformly over a finite interval ranging from zero to  $h_{\text{max}}^\sigma$  or  $h_{\text{max}}^\pi$ . We take  $h_{\text{max}}^\pi = 0.5h_{\text{max}}^\sigma$ . The energies are obtained by explicit diagonalization of a tight-binding Hamiltonian for this cluster. All of the ligand orbitals are taken to have the same value of  $\varepsilon_{L,\eta}$ . The only dimensionless variable that enters the results is then  $\gamma = |h_{\text{max}}/(\varepsilon_d - \varepsilon_{L,\eta})|$ . For small values of  $\gamma$ , the bonding is primarily ionic, and for larger values it acquires more covalent character. We use  $\gamma = 0.1$  for our results, but even when  $\gamma$  is significantly larger we find that the accuracy of the semiclassical form is essentially the same.

We have fitted the energies of these clusters to the following semiclassical form for the energy:

$$E_{\text{LFSE}}^2 = \sum_{L,\eta,L',\eta'} U(L,\eta;L',\eta') + \beta \quad , \quad (27)$$

involving two parameters  $\alpha$  and  $\beta$  (where  $U$  contains  $\alpha$ ). We use a database of 10,000 clusters to determine the parameters, and then test them on a set of 1000 clusters not included in the “training” set. Typical results are shown in Fig. 2, which shows the semiclassical energies vs. exact energies for 1000 clusters. Figure 2a corresponds to three  $\sigma$ -bonded ligands and one  $\pi$ -bonded one, while Fig. 2b corresponds to four  $\pi$ -bonded ligands. The rms errors in  $E_{\text{LFSE}}$  for these two cases are 9% and 14%, respectively. For clusters with two and three  $\pi$ -bonded ligands, the rms errors are 10% and 11%. This is to be compared with rms errors of 25%–30% that are obtained with empirical force fields (Carlsson, 1998).

#### IV. SMALL CLUSTER MINIMUM-ENERGY STRUCTURES

In this section, we describe some of the implications of the angular forms developed above for the structure of small model clusters consisting of a metal atom and four ligands. Such cluster calculations cannot treat the protein environment accurately; this would await parametrization and incorporation of the force field into protein codes, which is in progress in our group. However, from the small-cluster calculations it is possible to see the structural preferences of the angular forces by themselves. These are an important factor in the final structure adopted by a protein.

To keep the calculations as simple as possible, we place the ligands at frozen bond lengths from the central metal ion. Here “frozen means” that they are fixed at a given set of values, but these values are not necessarily the same for all of the ligands. The energy terms include the ligand-field stabilization energy as described by our angular terms, as well as a repulsive radial interaction between ligands forbidding close approaches. The latter term is needed because otherwise spurious structures involving  $45^\circ$  bond angles can appear when  $\pi$ -bonding ligands are present. For simplicity, we assume that the values of  $h_\sigma$  and  $h_\pi$  at a given distance are the same. To evaluate the magnitudes of these couplings, we assume a value of  $100 \text{ kJ/mole} = 23.9 \text{ kcal/mole} = 1.04 \text{ eV}$  for the ligand-field stabilization energy of a cluster with four  $\sigma$ -ligands in square coordination; this number would correspond to the more strongly stabilized complexes (Cotton, 1972). For clusters with unequal bond lengths, we assume an exponential decay, so that  $h_\sigma(r) = h_\sigma(r_0) \exp[-\kappa(r - r_0)]$ , where  $r_0$  is the reference distance and  $\kappa$  is a decay parameter. Since the most prominent case that we consider of a distant ligand is sulfur, we identify  $\kappa$  with the spatial decay rate coming from the measured first ionization energy  $E_1$  of sulfur,  $10.4 \text{ eV} (= 240 \text{ kcal/mole})$ , using the formula  $\hbar^2 \kappa^2 / 2m = E_1$ . This yields  $\kappa = 1.65 \text{ \AA}^{-1}$ . The ligand-ligand terms contain an exponential term taken as the repulsive part of the van der Waals interactions as given in the “MM2” force field (Sprague et al., 1987). In our model clusters, we use parameters and bond lengths typical for copper or nickel interacting with nitrogen and/or sulfur ligands. This is because copper and nickel have the largest ligand-field energies among the 3d transition metals, and typical ligands for these metals are nitrogen and sulfur. We have examined four simple geometries; the corresponding minimum-energy clusters are shown in Fig. 3.

a) A cluster with all four ligands  $\sigma$ -bonded and placed at equal distances of  $2.0 \text{ \AA}$ . We take repulsion parameters appropriate for nitrogen. The result is a square-planar cluster (*cf.* Fig. 3a), consistent with the observed structures of many copper and nickel complexes. With this parameterization, the square structure is quite strongly favored over the tetrahedral one, by  $0.31 \text{ eV}$  or  $7.0 \text{ kcal/mole}$ . The square structure remains stable under increases of the ligand-ligand repulsion strengths of up to a factor of over three. We note, however, that the stability of the square structure is strongly sensitive to the choice of repulsive terms. For example, we find that when the van der Waals term from the OPLS-II force field (Jorgensen et al., 1988) is used instead of the MM2 form, an increase of the repulsion strengths of only 20% is enough to stabilize the tetrahedral structure. We expect that input from fully quantum-mechanical total-energy calculations will be necessary to precisely pin down the competition between the electronic energy and the repulsion terms in determining the structural energy differences.

b) A cluster similar to that of a), but with one  $\pi$ -bonded ligand. This leads to a sub-

stantial deviation from planarity, as shown in Fig. 3b, where the  $\pi$ -bonded ligand is atom 4. As expected from the maximum of the  $\pi$ - $\sigma$  potential in Fig. 1, the angle between atom 4 and atoms 1–3 is greater than  $90^\circ$ , about  $123^\circ$ . The angle between the  $\sigma$ -bonded atoms 1, 2, and 3, is slightly greater than the ideal value of  $90^\circ$ , because of the repulsive energy term.

c) A cluster with with two  $\sigma$ -bonded nitrogen ligands 1 and 2, one  $\sigma$ -bonded sulfur ligand 3, and a  $\pi$ -bonded sulfur ligand 4. The distances are  $2.04\text{\AA}$  for the nitrogen ligands,  $2.18\text{\AA}$  for the  $\pi$ -bonded sulfur ligand, and  $2.64\text{\AA}$  for the  $\sigma$ -bonded sulfur ligand. This geometry is motivated by the observed geometry of copper sites in “blue-copper” proteins. In these proteins, the nitrogen ligands and a cysteine sulfur ligand are close to the copper, and it is believed that the cysteine sulfur ligand is primarily  $\pi$ -bonded. An additional  $\sigma$ -bonded methionine sulfur ligand is farther from the copper. The values of the ligand distances here are taken from cluster calculations for blue-protein models (Ryde et al., 1996). The van der Waals parameters are taken from the OPLS parameter set but we do not have a reliable procedure for determining the differences between the ligand-ion interactions of the nitrogen and the sulfur ligands. Since the greater distance of the sulfur at  $2.18\text{\AA}$  from the copper will be compensated to some extent by the greater size of sulfur relative to nitrogen, we simply assume that the three close ligands have the same coupling strength to the central atom. We have varied the ratio of the sulfur to nitrogen coupling strengths by up to 30% in both directions and found changes of only a few degrees in the bond angles of the cluster. For the far ligand, we use the scaling procedure described above, which leads to a coupling that has 50% of the strength of the close ligands. We have varied this coupling from 0% to 75% of the close-ligand value, and again found bond-angle changes of only a few degrees. The lowest-energy structure for this cluster is indicated in Fig. 3c. The geometry is trigonal, with bond angles of  $125^\circ$  between the cysteine sulfur and the nitrogens; the nitrogen-nitrogen bond angle is  $91^\circ$ . By comparison, the optimal values for protein models obtained by quantum-chemical calculations (Ryde et al., 1996) are  $125^\circ$  for the cysteine sulfur-nitrogen bond angle and  $103^\circ$  for the nitrogen-nitrogen bond angle. The results obtained by the present method are do of course not have quantitative accuracy, but the overall structure of the coordination shell is quite similar to that obtained in (Ryde et al., 1996), which is shown in Fig. 3d. The stability of this structure is not predetermined by the bond lengths that we used as input. To demonstrate this, we have considered a cluster with the same bond lengths as the blue-protein fragment, with with all ligands  $\sigma$ -bonded. In this case, the structure is planar, as shown in Fig. 3e. Thus the formation of the trigonal structure is directly related to the special character of the angular interactions associated with  $\pi$ -bonding.

## V. CONCLUSION

We have seen that it is possible to extract the functional form of angular forces around transition metals in biomolecules by using an approximate treatment of the ligand-field Hamiltonian. The approximations made here, involving primarily ionic bonding, are complementary to those used in (Landis et al., 1998). The resulting angular forms have simple trigonometric forms, which are very different for  $\pi$ -bonding as compared to  $\sigma$ -bonding. Ligand-field energies are represented well by these angular forces, and much better than by force fields with assumed functional forms. Future work should aim to include the functional forms derived here in widely used biomolecular simulation packages.

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## FIGURES

FIG. 1. (a) Interaction between ligands of central transition metal ion. The dimensionless quantity  $u$  (*cf.* Eqs. (19)–(21)) is either  $u_{\sigma,\sigma}$  (solid line),  $u_{\pi,\sigma}$  (dashed line), or  $u_{\pi,\pi}$  (dotted line). The quantity  $-u$  is plotted so that negative values will correspond to lower energies. (b) Empirical force field from (Comba et al., 1995).

FIG. 2. Energies obtained by semiclassical force field vs. exact energies for small model clusters. Energies given in units of  $h_{\max}$ , maximal coupling strength between ligands and transition metal. (a) One  $\pi$ -bonded ligand and three  $\sigma$ -bonded ligands. (b) Four  $\pi$ -bonded ligands.

FIG. 3. Lowest-energy structures obtained in relaxations of five-atom cluster using angular forces. (a) Four equivalent  $\sigma$ -bonded neighbors. (b) Three  $\sigma$ -bonded neighbors and one  $\pi$ -bonded neighbor 4, all at the same distance. (c) Two  $\sigma$ -bonded nitrogen neighbors 1 and 2, a near  $\pi$ -bonded sulfur neighbor 3, and a far  $\sigma$ -bonded neighbor 4. (d) Structure for blue protein model obtained by quantum-chemical calculations (Ryde et al., 1996). (e) Three near  $\sigma$ -bonded neighbors and a far  $\sigma$ -bonded neighbor.

















